Sex and segregation in temperate bats

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Many temperate insectivorous bats show marked sexual segregation during the summer, but in spectacular, pre-hibernation swarming, gather at caves to mate. In many species, sexual segregation is probably due to a gradient in aerial insect availability that confines females to lower elevations, where high reproductive costs are met by an abundant and reliable food supply. In the hawking and trawling *Myotis daubentonii*, we show that alongside inter-sexual segregation, there is intra-male segregation and suggest that this results from the exclusion of most males from high-quality habitat. These apparently excluded males suffer reduced foraging efficiency and mating success relative to males that roost with the females in summer. Changes in resources and behaviour at the end of the summer lead to a change in strategy that gives all males a chance to mate during swarming, but this does not overcome the paternity advantage to males that spend the summer with the females.

Keywords: bats; mating; paternity; sexual segregation; swarming

1. INTRODUCTION

Sexual segregation is widespread in mammals and numerous studies have proposed and tested hypotheses that might explain it (Ruckstuhl & Neuhaus 2000). The most important causal mechanisms appear to be related to sex-based differences in activity budgets, foraging requirements or social behaviour, but these are hotly debated (Ruckstuhl & Neuhaus 2002; Bonenfant *et al.* 2004). However, most of this work has been carried out on sexually dimorphic ungulates, in which the difference in size is central to the segregation mechanism. We have studied inter- and intra-sexual segregation in a member of the diverse and speciose bats, in which sexual dimorphism is rare, but segregation is widespread.

Daubenton's bat, *Myotis daubentonii*; is a small insectivorous species widespread in Europe. In summer it feeds almost exclusively over flat water, catching insects from the air or gaffing them from the surface with its feet. It roosts in holes in bank-side trees or in the stonework of bridges. In the late summer and autumn, nursery colonies disperse and the bats visit caves and disused mines, where many eventually hibernate (Altringham 2003). Prior to hibernation, in a behaviour pattern known as swarming, the caves are used as assembly points and probably as mating sites by large, transient bat populations from substantial catchment areas (Kerth *et al.* 2003; Parsons *et al.* 2002). We studied a ringed population in the Yorkshire Dales National Park, UK. Over a six-year period, with one exception, only males were caught high in the dale, but another colony of males was found with females at lower elevations. We present evidence showing that these two colonies do not mix during the summer and test hypotheses to explain this segregation.

We reasoned that the absence of females at higher elevations, a widely observed phenomenon across bat

species (Barclay 1991; Leuzinger & Brossard 1994; Cryan *et al.* 2000), could be explained by the high energetic demands of pregnancy and lactation, which could not be met by sub-optimal foraging conditions upstream. Evidence comes from work on related species, *Myotis lucifugus* and *Myotis evotis*, in Canada (Barclay 1991). In the foothills of the Canadian Rockies, male *M. lucifugus* dominated the summer population; only 7% of the bats caught were females and less than half of these were pregnant. The majority of the females formed large nursery colonies at low elevations. This species, like *M. daubentonii*, fed primarily over water on aerial prey that were abundant only for a short time after sunset at high elevation; at night the temperature frequently fell below the minimum flight temperature for many insects (Taylor 1963; Peng *et al.* 1992). The argument that low temperature restricted female *M. lucifugus* to low elevations was strengthened by parallel studies, at the same study site, on the related species *M. evotis:* 44% of captured *M. evotis* were females and over one-third of these were either pregnant or lactating. This species fed primarily by gleaning non-flying insects from vegetation and females were able to obtain sufficient food even at low temperatures, when few insects were flying (Barclay 1991). Recent studies of *M. daubentonii* in Europe (Leuzinger & Brossard 1994) and 11 species of insectivorous bat in the Black Hills of South Dakota (Cryan *et al.* 2000) support the finding that the females of many aerial insectivorous bats are unable to reproduce in marginal upland habitats.

Facultative heterothermy (torpor) is widely used by temperate bats and even a moderate reduction in core temperature can lead to substantial energy savings (Speakman & Thomas 2003). Reproductive females make less use of torpor, since it reduces foetal growth rates and possibly milk production (Racey & Entwistle 2000). However, in the summer, males have lower energy requirements and they are able to use torpor when foraging conditions are poor (Hamilton & Barclay 1994). This explains their ability to exploit the entire river system. But why are there two discrete summer colonies of males? Two hypotheses were considered.

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Table 1. Foraging statistics determined from radiotelemetry.

(F, female; DSM, downstream male; USM, upstream male. Data are expressed as mean (s.d.). Statistically significant results are shown in bold. Maximum foraging distance is the maximum distance flown between the roost and a foraging site each night. Time out of roost is expressed as % of darkness hours. When expressed in absolute time (hours), a similarly significant result was obtained. Differences over all traits were highly significant (MANOVA: Hotelling's Trace $F = 4.012$; df = 10, 12; $p = 0.013$).)

	mean (s.d.)			between group effects			*post hoc* tests (LSD)		
	female	downstream male	upstream male	d.f.	F	sig.	F versus DSM	F versus USM	DSM versus USM
n	6	4	4						
emergence time after sunset (min.)	40.2 (10.5)	53.4 (10.8)	43.8 (3.8)	2,11	2.505	0.127			
max. foraging distance (m)	1688 (844)	1225 (384)	6513 (6220)	2,11	6.053	**0.017**	0.63	**0.013**	**0.009**
time out of roost as % of night length	65.3 (8.9)	62.5 (4.5)	78.8 (6.5)	2,11	6.683	**0.013**	0.567	**0.011**	**0.007**

(i) Upstream males are excluded from the better downstream habitat by dominant males and/or by females that share roosting and foraging sites. If correct, this hypothesis predicts that upstream males will find it harder to obtain food and will have lower mating success than downstream males.

(ii) Upstream males are better-suited to the upstream environment and are simply avoiding competition with downstream bats. They do not need summer access to females, since mating occurs at swarming sites later in the year. Mating success will, therefore, be comparable to that of downstream males.

These alternatives reflect a general dichotomy in theories of sexual segregation which may be due to exclusion of one sex from a universally favourable environment or to segregation into different environmental compartments that suit the needs of one or the other sex. To test these hypotheses we compared the foraging behaviour and condition of the upstream males with those of downstream males and females. Mating success was studied using microsatellite-based paternity analysis.

2. MATERIAL AND METHODS

(a) *Study site*

The study was performed on a 20 km stretch of the River Wharfe in the Yorkshire Dales National Park, UK. Over this stretch, the river falls from 250 to 150 m a.s.l., widens from 3–10 m to over 20 m and becomes smoother, with fewer sections of fast-flowing and noisy water, over which the bats do not feed (Warren *et al.* 2000) due to impaired echolocation performance (Rydell *et al.* 1999). The narrow upstream river, with its small flat-water pools and overhanging vegetation is expected to increase foraging flight costs due to the need for frequent turns. Furthermore, mean nightly summer temperature is lower, and variation is significantly greater at the higher elevations (Turner 2002) and temperature falls below the critical temperature for insect flight on more nights (Taylor 1963; Peng *et al.* 1992; Turner 2002). Both bat activity and flying insect density were positively correlated with temperature over the river (Turner 2002) and the upstream environment may not provide the regular and abundant insect supply demanded by the females.
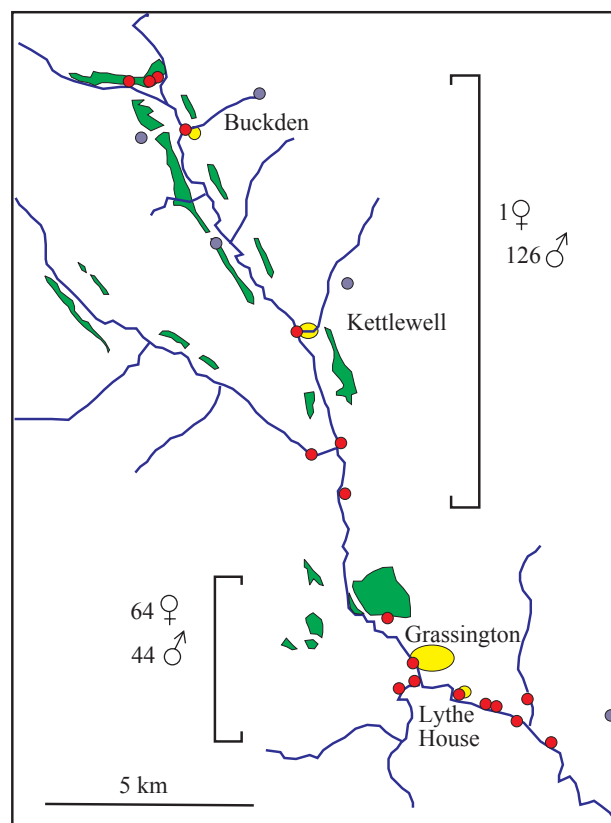

Figure 1. The study site in the upper reaches of the River Wharfe in the Yorkshire Dales National Park. Summer roosts (red circles) and swarming sites/hibernacula (blue circles) are shown. The figures refer to the number of adult bats caught and ringed from the roosts and foraging sites during the summer months. The primary roosts are the bridges at Buckden and Kettlewell (upstream males) and Grassington (nursery) and a tree roost at Lythe House (nursery). All other roosts are satellites of these. All sampling was done at the four primary roosts.

(b) *Capture and processing*

Bats were captured and ringed (April–November inclusive) under an English Nature licence, using mist nets and harp traps and static hand nets. Biopsy punches (3 mm diameter) were taken from both wings under Home Office and English
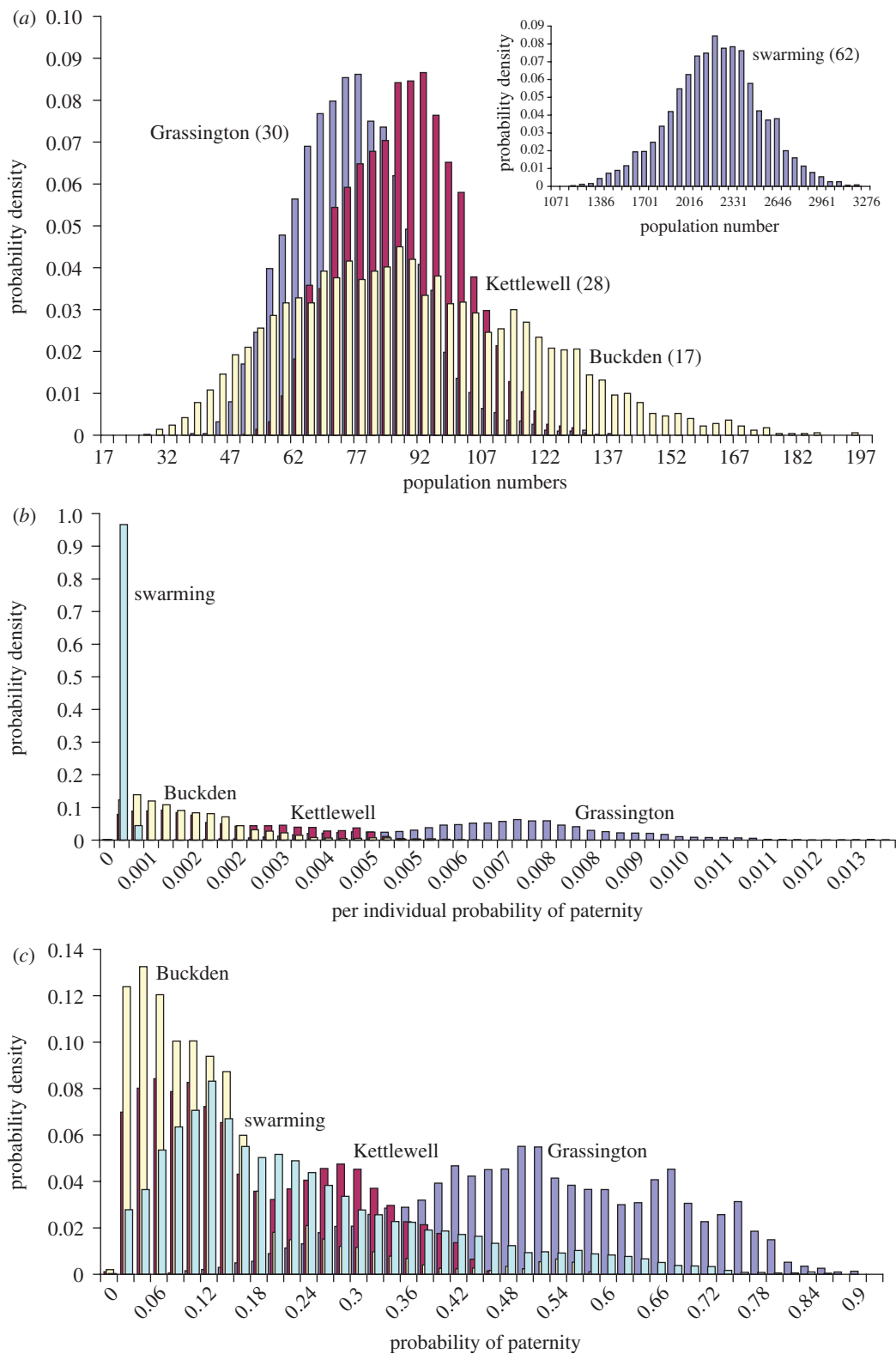
Figure 2. (*a*) Estimated numbers of potential male parents, *Myotis daubentonii*, at Grassington (downstream), Kettlewell and Buckden (upstream) bridge roosts and at the swarming sites (inset). The numbers of genotyped individuals are shown in parentheses. The estimates include turnover for the three-year study period. (*b*) Probability of an individual male from each of the roosting groups or the swarming sites fathering a juvenile bat from the Grassington nursery colony. (*c*) The roosting group level probability of paternity of the Grassington colony offspring.

Nature licences. Body mass and forearm length were measured for all bats. Forearm length is widely used as an easy-to-measure indicator of skeletal size. Mass/forearm length ratio or body condition index (BCI; Speakman & Racey 1986) has been used as a measure of body condition, the higher BCI, the better the condition of the bat.

Wing size and shape parameters of weighed bats (Norberg 1990) were determined from digital photographs of a sample of bats captured during a two-week period in July 2000. The right wing and tail membrane were extended with the bats face down on a flat surface and a digital photograph taken (Nikon E3s camera) from a fixed distance directly above the bat.

(c) Radiotelemetry

Radio transmitters (Holohil LB2: 0.44–0.48 g; <6% body weight) were fitted under an English Nature licence. Bats were tracked one at a time (May–August inclusive), from departure from the roost until their return. Only bats followed for a minimum of four complete nights after the night of capture were included in the analysis. Data were used only if radio contact was maintained for a minimum of 95% of the time bats were out of the roost. Loss of contact in these cases was brief and due to the time it took to catch up with commuting bats. Since the bats flew only over the river, the position of a bat could be estimated to within 20 m except when the bats were commuting. Visual contact could be made throughout using a night scope. A continuous record of position and behaviour was made, typically by two trackers.

(d) Population estimates and genetics

DNA was extracted from the wing membrane samples using a phenol–chloroform protocol. Ten microsatellite loci were amplified using primers developed in other bat species (Burland *et al.* 1998; Castella & Ruedi 2000; Kerth *et al.* 2002), including an X-linked locus (table 1 in the electronic supplementary material). PCR products were separated on an ABI377 sequencer and sized using GENESCAN and GENOTYPER software (Applied Biosystems, Warrington). Since offspring could not be caught with their mothers in the inaccessible roosts, bats were captured on emergence. A model was required that would assign both maternity and paternity. However, we were concerned not with the identities of individual parents but with the probabilities that fathers were drawn from one of four male groups: two upstream roosts (part of a single colony), downstream roost or swarming site males. A Bayesian approach developed for a similar problem in a different bat species (Burland *et al.* 2001) was adapted to take into account the single female colony and the four male groups, allowing for the fact that males from the dale contribute to the group at swarming sites. The probabilities of the parental combinations were calculated using simple adaptations of the formulae in the Appendix to Burland *et al.* (2001) and these are described in the electronic supplementary material.

The paternity analysis requires estimates of the numbers of bats in the male and female roosts and at swarming sites. These were obtained from recaptures of ringed bats over an eight-year period, using methods described in the electronic supplementary material, and entered into the paternity analysis as prior probability distributions.

3. RESULTS

(a) Segregation and swarming

Over a six-year period only one of 127 bats caught and ringed in the upper dale was a (foraging) female (figure 1). The sex-ratio of all adult bats caught in the lower dale, including those caught in roosts was 2.6 : 1, female : male (figure 1). Of 176 adult male recaptures, only one upstream male made one visit to a downstream roost. In over 150 nights of radiotracking, no upstream bat flew to the downstream locations or vice versa. Segregation was not based on age; all roosting groups showed similar age class profiles of 1 to >8 year old bats (minimum age, based on ringing date, data not shown; Senior 2005).

Of the 62 bats caught at the swarming sites shown in figure 1, two were upstream males and five downstream males. More than 80% of both adult and juvenile bats were males. No bats were captured at more than one swarming site. Only a small proportion of bats (<5%; Rivers *et al.* in press) visit more than one site, limiting the mating opportunities of the males during swarming. However, most bats captured at swarming sites had not previously been caught in the study area, a reflection of the large, transient populations of males characteristic of swarming (Parsons *et al.* 2002; Rivers *et al.* in press). Roosting group size estimates from mark–recapture analysis (see electronic supplementary material) showed that we had sampled a high proportion of the potential upstream (Buckden and Kettlewell) and downstream (Grassington) males (170 ringed out of approximately 250), but only a small proportion of those visiting swarming sites (figure 2a).

(b) Radiotelemetry

Bats were tracked for 4–13 consecutive nights, from emergence to return to the roost. There were no significant differences in mean emergence time between up- and downstream males and females. However, upstream males spent significantly more time out of the roost, and travelled to more distant foraging sites, than either females or downstream males (table 1). All bats occupied short (<100 m) foraging 'beats' on the river, to which they returned night after night. Each bat had 2–3 foraging sites, but upstream males tended to spend less time at their main site than the others. Downstream males foraged within the home range of the tracked females and often occupied beats very close to the roost. All bats were more faithful to their foraging sites than their roost sites: they switched roosts on average every three days and most bats used at least two roosts during the brief period they were tracked, suggesting that roosts were not a limiting resource but foraging beats were.

Combined night roosting and commuting times were short and similar for all groups, averaging just 20 min night^{-1} combined.

(c) Morphology

Females were slightly but significantly larger and heavier than males (table 2). Despite their similar forearm length, upstream males were significantly lighter than downstream males, contributing to a significantly lower BCI, and a lower wing loading (body weight/wing area).

(d) Population estimates and genetics

Roosting group sizes are shown in figure 2a, based on data from 120 recaptures, on 54 sampling occasions, over 8 years, during which a total of 218 male bats were marked (151 of which were used in the population estimate, those caught at the primary roosts). Roosting group size priors for the paternity analysis were based on sizes adjusted for turnover rates during the period of offspring sampling to reflect the numbers of potential parents (see electronic supplementary material). Figure 2b shows the probability that a male from each of the four male roosting groups will be a father, based on 38 juveniles captured from downstream nursery roosts. These represent an estimated 32% of the colony offspring over the three years of sampling (assuming all females produce one offspring each year or 40% assuming 80% reproduce each year; downstream males had the highest individual probability of paternity in more than 94% of iterations after burn-in, see electronic

Table 2. Morphological characteristics of upstream males (Kettlewell) and downstream males and females (Grassington), from a sample of bats caught over two weeks in July 2000.
(F, female; DSM, downstream male; USM, upstream male. Data are expressed as mean (s.d.). Statistically significant results are shown in bold. Aspect ratio and the three tip indices reflect the shape of the wing. Since no differences were found in these, they are not discussed further. (MANOVA including all traits: Hotelling's Trace $F = 7.154$; df $= 10, 112$; $p < 0.001$).)

	mean (s.d.)			between group effects			*post hoc* tests (LSD)		
	female	downstream male	upstream male	d.f.	F	sig.	F versus DSM	F versus USM	DSM versus USM
n	24	11	29						
forearm length (mm)	37.3 (0.8)	36.7 (1.1)	36.8 (0.7)	2,61	3.406	**0.04**	**0.038**	**0.027**	0.684
mass (g)	8.4 (0.8)	7.9 (0.7)	7.3 (0.4)	2,61	19.784	**<0.001**	**0.024**	**<0.001**	**0.014**
mass/forearm length ratio	0.226 (0.019)	0.215 (0.017)	0.199 (0.012)	2,61	18.992	**<0.001**	0.068	**<0.001**	**0.006**
tip length ratio	1.21 (0.049)	1.22 (0.098)	1.24 (0.093)	2,61	0.915	0.406			
tip area ratio	0.57 (0.048)	0.57 (0.065)	0.59 (0.057)	2,61	0.597	0.554			
wing loading (N m^{-2})	8.8 (0.9)	9.1 (1.1)	8.2 (0.7)	2,61	5.041	**0.009**	0.398	**0.018**	**0.007**
aspect ratio	6.8 (0.4)	6.6 (0.3)	6.8 (0.3)	2,61	0.821	0.445			
tip index	0.90 (0.125)	0.88 (0.123)	0.90 (0.098)	2,61	0.15	0.861			

supplementary material for details). Figure 2c shows the probability of paternity of the same offspring at the roosting group level.

The paternity analysis was conducted with the priors from these analyses, with shallower prior distributions and with flat prior distributions. Higher and lower microsatellite mutation/scoring-error rates were also investigated. In all cases, the qualitative result was the same; downstream males had the highest mating success per male.

A two-step, individual analysis of parentage was conducted using the likelihood-based statistical program CERVUS (Marshall *et al.* 1998). The first step was to assign maternity to the 38 juveniles from the Grassington roost. Ringing data showed that the same females used both the Grassington Bridge and Lythe House roosts, so data from the two roosts were combined. Maternity was assigned to 17 of the 38 offspring (45% maternity assignment), 13 at the 95% confidence level and four at the 80% confidence level. Of the 17 offspring with identified mothers, fathers were assigned to four (24% paternity assignment), one at the 95% confidence level and three at the 80% level; all of the fathers were from the Grassington roost. This level of assignment is compatible with the results of the Bayesian analysis, which estimates approximately 50% of the fathers to come from the Grassington roost where 36% of the estimated number of males in the Grassington roost have been genotyped. The assignment of all fathers to the Grassington roost supports the conclusion that males in this roost have a greater probability of paternity than upstream males.

4. DISCUSSION

The longer foraging times, use of more distant foraging sites and the low BCI are all consistent with the hypothesis that upstream males must work harder for their food in a poor habitat. However, an alternative explanation is that upstream males regulate their body mass to maintain low wing loading, as suggested for birds (Witter & Cuthill 1993). Lowering wing loading reduces flight costs and increases manoeuvrability (Norberg 1990). Both would be an advantage to upstream males. Lower flight costs would compensate for the increased cost of their longer foraging time and increased manoeuvrability would suit the more cluttered upstream foraging habitat. An explanation based on mass regulation is, therefore, consistent with the idea that upstream males are well suited to their environment, rather than making the best of a bad job.

To distinguish between the alternatives of poor body condition (consistent with hypothesis (i) in the §1) and adaptive mass regulation (consistent with hypothesis (ii)) requires a measure of body condition independent of mass and measurement of the real benefits of mass regulation. However, these are proximate measures of the ultimate goal: increased reproductive success. We have taken a more direct approach to the measurement of fitness and asked the question: do upstream and downstream males have an equal probability of fathering offspring? The results show clearly that mating is heavily skewed towards downstream males both in terms of the average probability of fathering an offspring for individual males (figure 2b) and the overall contribution of this group of males (figure 2c). However, a significant minority of offspring are fathered by both upstream and swarming site males.

The simplest interpretation of these results is that dominant males and/or females exclude the majority of males from the better foraging habitat around the nursery roosts. The dominant males are able to monopolize females in the late summer when they become receptive (Racey & Entwistle 2000). In common with most temperate species, female Daubenton's bats store sperm over winter and ovulation and fertilization occur in spring. Mating may take place from August to April, making mate guarding practically impossible and providing the opportunity for sperm competition. Mating by dominant males in the nursery roost may give them an advantage if their sperm are the first to secure a place in the oviduct, where they may be nourished through the winter (Racey & Entwistle 2000). Alternatively, or

additionally, dominant males may out-compete other males at swarming sites. During swarming, large numbers of bats chase or follow each other in and around the underground sites (Thomas *et al*. 1979; Parsons *et al*. 2002), mating on the walls of the cave or mine (Thomas *et al*. 1979). About 80% of bats captured at swarming sites are males (Parsons *et al*. 2002; Rivers *et al*. in press), suggesting intense competition for mates. There is some evidence to show that mating is skewed in favour of a smaller group of males (Watt & Fenton 1995). The sex ratio probably reflects behavioural differences between the sexes. Swarming must be energetically expensive, depleting food reserves prior to hibernation. Females that have successfully mated may visit swarming sites less frequently than males, for less time, or not at all. All males should seek to secure as many matings as possible and should, therefore, go to swarming sites as often as possible.

Recent work (Kerth *et al*. 2003; Kerth & Morf 2004; Veith *et al*. 2004) has highlighted the importance of swarming as a mating strategy in many temperate bats. In our study population most successful matings appear to occur before swarming, unless the dominant males successfully out-compete an overwhelming number of rivals at swarming sites. The small proportion of potential fathers sampled at swarming sites could have led to an underestimate of the proportion of successful swarming males. This would not alter the conclusion that downstream males secure more matings than upstream males, but raises some uncertainty about the relative importance of the two mating strategies to the wider population.

In conclusion, we provide evidence that segregation in the male population at summer home ranges is due to the exclusion of a large proportion of the males, leading to skewed male mating success. It is this exclusion, rather than differential resource use by males and females, that drives the overall sexual segregation. This mechanism may explain segregation in many other mammals but remains largely unexplored, perhaps because of the emphasis on ungulates in most previous studies. The resource priorities of the females change at the end of the summer, as they seek out underground hibernation sites, leading to changes in behaviour in both females and males. The consequence is a change in mating site and system, allowing other males to compete for females during swarming.

REFERENCES

Altringham, J. D. 2003. *British bats*. London: Harper Collins.

Barclay, R. M. R. 1991 Population structure of temperate zone bats in relation to foraging behaviour and energy demand. *J. Anim. Ecol.* **60**, 165–178.

Bonenfant, C., Loe, L. E., Mysterud, A., Langvatn, R., Stenseth, N. C., Gaillard, J. M. & Klein, F. 2004 Multiple causes of sexual segregation in European red deer: enlightenments from varying breeding phenology at high and low latitude. *Proc. R. Soc. B* **271**, 883–892. (doi:10.1098/rspb.2003.2661.)

Burland, T. M., Barratt, E. M. & Racey, P. A. 1998 Isolation and characterization of microsatellite loci in the brown long-eared bat, *Plecotus auritus*, and cross-species amplification within the family Vespertilionidae. *Mol. Ecol.* **7**, 136–138.

Burland, T. M., Barratt, E. M., Nichols, R. A. & Racey, P. A. 2001 Mating patterns, relatedness and the basis of natal philopatry in the brown long-eared bat, *Plecotus auritus*. *Mol. Ecol.* **10**, 1309–1321. (doi:10.1046/j.1365-294X.2001.01273.x.)

Castella, V. & Ruedi, M. 2000 Characterization of highly variable microsatellite loci in the bat *Myotis myotis* (Chiroptera: Vespertilionidae). *Mol. Ecol.* **9**, 1000–1002. (doi:10.1046/j.1365-294x.2000.00939-6.x.)

Cryan, P. M., Bogan, M. A. & Altenbach, J. S. 2000 Effect of elevation on distribution of female bats in the Black Hills, South Dakota. *J. Mammal.* **81**, 719–725. (doi:10.1644/1545-1542(2000)081<0719:EOEODO>2.3.CO;2.)

Hamilton, I. A. & Barclay, R. M. R. 1994 Patterns of daily torpor and day-roost selection by male and female big brown bats (*Eptesicus fuscus*). *Can. J. Zool.* **72**, 744–749.

Kerth, G. & Morf, L. 2004 Behavioural and genetic data suggest that Bechstein's bats predominantly mate outside the breeding season. *Ethology* **110**, 987–999. (doi:10.1111/j.1439-0310.2004.01040.x.)

Kerth, G., Mayer, F. & Petit, E. 2002 Extreme sex-biased dispersal in the communally breeding, nonmigratory Bechstein's bat (*Myotis bechsteinii*). *Mol. Ecol.* **11**, 1491–1498. (doi:10.1046/j.1365-294X.2002.01528.x.)

Kerth, G., Kiefer, A., Trappmann, C. & Weishaar, M. 2003 High gene diversity at swarming sites suggest hot spots for gene flow in the endangered Bechstein's bat. *Cons. Gen.* **4**, 491–499. (doi:10.1023/A:1024771713152.)

Leuzinger, Y. & Brossard, C. 1994 Repartition de *M. daubentonii* en fonction du sexes et de la periode de l'annee dans le Jura Bernois. *Mitt. Natf. Ges. Schaffhausen* **39**, 135.

Marshall, T. C., Slate, J., Kruuk, L. E. B. & Pemberton, J. M. 1998 Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* **7**, 639–655. (doi:10.1046/j.1365-294x.1998.00374.x.)

Norberg, U. M. 1990. *Vertebrate flight*. Berlin: Springer.

Parsons, K. N., Jones, G., Davidson-Watts, I. & Greenaway, F. 2002 Swarming of bats at underground sites in Britain—implications for conservation. *Biol. Cons.* **111**, 63–70. (doi:10.1016/S0006-3207(02)00250-1.)

Peng, R. K., Fletcher, C. R. & Sutton, S. L. 1992 Effect of microclimate on flying diptera. *Int. J. Biomet.* **36**, 69–76. (doi:10.1007/BF01208916.)

Racey, P. A. & Entwistle, A. C. 2000 Life-history and reproductive strategies of bats. In *Reproductive biology of bats* (ed. E. G. Crichton & P. H. Krutzsch), pp. 363–414. London: Academic Press.

Rivers, N. M., Butlin, R. K. & Altringham, J. D. In press. Autumn swarming behaviour of Natterer's bats in the UK: population size, catchment area and dispersal. *Biol. Cons.*

Ruckstuhl, K. E. & Neuhaus, P. 2000 Sexual segregation in ungulates: a new approach. *Behaviour* **137**, 361–377. (doi:10.1163/156853900502123.)

Ruckstuhl, K. E. & Neuhaus, P. 2002 Sexual segregation in ungulates: a comparative test of three hypotheses. *Biol. Rev.* **77**, 77–96.

Rydell, J., Miller, L. A. & Jenson, M. E. 1999 Echolocation constraints of Daubenton's bat foraging over water. *Funct. Ecol.* **13**, 247–255. (doi:10.1046/j.1365-2435.1999.00304.x.)

Senior, P. 2005 Sexual and spatial segregation in Daubenton's bat (*Myotis daubentonii*). Ph.D. thesis, University of Leeds.

Speakman, J. R. & Racey, P. A. 1986 The influence of body condition on sexual development of male brown long-eared bats (*Plecotus auritus*) in the wild. *J. Zool. Lond.* **210**, 515–525.

Speakman, J. R. & Thomas, D. W. 2003 Physiological ecology and energetics of bats. In *Bat ecology* (ed. T. H. Kunz & M. B. Fenton), pp. 430–490. Chicago: University of Chicago Press.

Taylor, L. R. 1963 Analysis of the effects of temperature on insect flight. *J. Anim. Ecol.* **32**, 99–117.

Thomas, D. W., Fenton, M. B. & Barclay, R. M. R. 1979 Social behaviour of the little brown bat, *Myotis lucifugus*. I. Mating behaviour. *Behav. Ecol. Sociobiol.* **6**, 129–136. (doi:10.1007/BF00292559.)

Turner, V. G. T. 2002 Aspects of the ecology of Daubenton's bats and pipistrelles and their potential prey in relation to altitude. Ph.D. thesis, University of Leeds.

Veith, M., Beer, N., Kiefer, A., Johannesen, J. & Seitz, A. 2004 The role of swarming sites for maintaining gene flow in the brown long-eared bat (*Plecotus auritus*). *Heredity* **93**, 342–349. (doi:10.1038/sj.hdy.6800509.)

Warren, R. D., Waters, D. A., Altringham, J. D. & Bullock, D. J. 2000 The distribution of Daubenton's bats (*Myotis daubentonii*) and pipistrelle bats (*Pipistrellus pipistrellus*) (Vespertilionidae) in relation to small-scale variation in riverine habitat. *Biol. Cons.* **92**, 85–91. (doi:10.1016/S0006-3207(99)00062-2.)

Watt, E. M. & Fenton, M. B. 1995 DNA fingerprinting provides evidence of discriminate suckling and non-random mating in little brown bats *Myotis lucifugus*. *Mol. Ecol.* **4**, 261–264.

Witter, M. S. & Cuthill, I. C. 1993 The ecological costs of avian fat storage. *Phil. Trans. R. Soc. B* **340**, 73–92.